Adenosylcobalamin-dependent Rearrangement Reactions: Model Studies of Diol Dehydratase

By Richard G. Finke* and William McKenna
(*Department of Chemistry, University of Oregon, Eugene, Oregon* 97403)

Summary A direct probe of the key putative cobalt–carbon bound diol dehydratase intermediate (**1**) has been provided by the preparation of (**2a**), a carbonate-protected B_{12} model form of (**1**) and by its MeO^--catalysed deprotection to yield ethanal and other products.

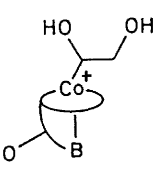
Enzymes requiring coenzyme B_{12} as a cofactor, such as diol dehydratase (equation 1), catalyse eleven unusual rearrangement reactions. The precise mechanism of these reactions continues to attract considerable interest and effort.[1]

$$\mathrm{RCHOH{-}CH_2OH} \xrightarrow{\text{diol dehydratase}} \mathrm{RCH_2CHO + H_2O} \quad (1)$$

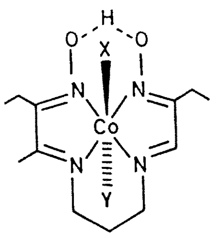
(R = Me or H)

Studies of diol dehydratase and the other B_{12}-dependent enzymes have provided evidence for a mechanism involving the initial production of radicals[2] *via* cobalt–C(5′) bond homolysis followed by the participation of the C(5′) radical as an obligatory H• transfer site.[3] Studies of the enzymes have failed, however, to provide any details about the subsequent rearrangement (*e.g.* OH migration) step (or steps),[4] and recent model studies[5] have striven to provide chemical precedent for the mode (or modes) of rearrangement.

Previous model diol dehydratase studies have often involved photolysis[6] of cobalt-alkyls in the presence of intra- or inter-molecular glycols to yield, among others, some glycol rearrangement products. Although these photolysis studies provide a precedent for the H• transfer step in diol dehydratase, the role of cobalt, if any, in the steps following the initial photocleavage of the Co–C bond is unclear. Model studies consistent with the participation of cobalt(III) π-complexes in diol dehydrase rearrangements have appeared.[7] The participation of Co^{III} π-complexes has, however, never been directly tested since a model containing OH groups α to cobalt, as in the key putative cobalamin intermediate (**1**) has, heretofore, never been prepared.[8]



(1)



(2)

Compound	X	Y
(**2a**)	$-\mathrm{CH{\cdot}O{\cdot}C(:O){\cdot}O{\cdot}CH_2}$	Cl
(**2b**	CO	—
(**2c**)	Cl	—
(**2d**)	$\mathrm{Bu^n}$–	Br
(**2e**)	$\mathrm{MeOCH_2}$–	Cl
(**2f**)	$\mathrm{CH_2(OH)CH(OH)}$–	Cl
(**2g**)	$-\mathrm{CH_2CHO}$	Cl
(**2h**)	Cl	Cl

We now report the high-yield synthesis, isolation, and characterization [including 360 MHz n.m.r. spectrum] of (**2a**), a model precursor to (**1**) where the glycol is protected as the carbonate, as well as a preliminary study of its base-catalysed deprotection and resulting reaction. We chose to use Costa's corrin model[9a] (**2**) because of our recent electrochemical comparative[9b] study demonstrating that this model most closely reproduces the known electrochemistry of B_{12}.

Oxidative-addition under N_2 of a 5—10 fold excess of chloroethylene carbonate[10] to a blue benzene solution of the cobalt(I)–CO compound[9c] (**2b**) yields[11] (**2a**) [330 mg, orange ppt.]. Preparative t.l.c. in air (SiO_2, acetone) yields another 220 mg to give a combined crude yield of 80%. Recrystallization (EtOH–acetone in air) yields 410 mg of analytically pure (**2a**) which has been characterized by elemental analysis (satisfactory for $C_{16}H_{26}N_4O_5CoCl$), a preliminary *X*-ray diffraction study,† and i.r. [ν_{CO}(KBr) 1778 cm^{-1}] and 360 MHz ^1H n.m.r. spectroscopy.‡ The 360 MHz ^1H n.m.r. spectrum was required to resolve the numerous sets of diastereotopic hydrogens resulting from the chiral carbon centre attached directly to cobalt

In a control experiment, treatment of a 0·022 M ethylene carbonate–MeOH solution with 1 equiv of MeO^- catalyst yields 1 equiv each of ethylene glycol (in CD_3OD as $DO[CH_2]_2OD$, δ 4 5, s) and dimethyl carbonate [ν_{CO}(MeOH) 1755 cm^{-1}] as rapidly (< 1 min) as the reaction can be monitored by i r or n m r spectroscopy Similarly, the treatment of an orange, homogeneous 0 022 M solution of (**2a**) in MeOH[§] with 1 equiv of MeO^- results in rapid formation ($t_{1/2}$ < 1 min) of a homogeneous, burgundy-coloured solution [λ_{max} (MeOH) 520 nm] identical to that formed in an independent preparation[¶] of authentic (**2c**) (Co^{II}) Using a combination of i r , e s r , and visible spectroscopy, as well as g l c we have established[**] the nearly complete reproducible stoicheiometry shown in equation (2)

$$(\mathbf{2a}) + MeO^-(MeOH,\ N_2) \longrightarrow (\mathbf{2c})\ (Co^{II}) + \tfrac{1}{2}\,MeCHO + \tfrac{1}{2}\,MeOCO_2^- + \tfrac{1}{2}\,MeOC(\ O)OMe \quad (2)$$

N m r experiments (CD_3O^-,CD_3OD) show that essentially no (⩽ 5%) $HO[CH_2]_2OH$ or $HOCH_2CHO$ is formed If degassed, orange, 0·022 M MeOH solutions of cobalt-alkyls lacking the carbonate group, such as (**2d**) or (**2e**), are treated with 1 equiv of MeO^-, no colour change is observed. This lack of Co^{II} formation with (**2d**) and (**2e**) and the control showing ready methanolysis of ethylene carbonate strongly suggests that the production of Co^{II} in equation (2) is the result, as expected, of initial MeO^- reaction with (**2a**) at its carbonate carbonyl group The presence of *ca* 1/2 equiv each of $MeOCO_2^-$ and $(MeO)_2CO$ suggests by mass balance that *ca* 1/2 equiv each of (**2f**) and possibly (**2g**) are formed in this reaction prior to the formation of Co^{II} (**2c**) and, presumably, organic radicals such as $\cdot CH_2CHO$ Interestingly, when (**2a**) was treated with 1 equiv of MeO^- in MeOH in the presence of 1 equiv of the radical traps t-butylphenylnitrone[12] or the nitroxide radical trap[13] 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl (**3**), significantly lower MeCHO yields (⩽ 10%) were obtained A control experiment showed that acetaldehyde, present as its hemiacetal, is not consumed in the timescale of these experiments by 1 equiv of either $PhCH{=}N(O)CMe_3$ or (**3**) in the presence of 1 equiv of MeO^- in MeOH

The above results suggest that protected model forms of putative coenzyme B_{12} intermediates can serve as useful mechanistic probes Additional kinetic and mechanistic experiments with (**2**) and its B_{12} analogue will be reported shortly

We thank the National Institute of Health for a grant, the Biomedical Research support grant programme for funds to purchase an inert atmosphere box, the Petroleum Research Fund, and the Office of Scientific and Scholarly Research of the University of Oregon for a Faculty Research Award The 360 MHz n m r spectra were obtained at the Stanford Magnetic Resonance Lab with the expert assistance of Lois Durham

(*Received*, 1*st November* 1979, *Com* 1163)

[†] Crystals of (**2a**) were grown from MeCN by hexane vapour diffusion and were free of MeCN solvate by C,H,N analysis Space group $P2_1/M$ was determined by precession photography, a = 11 927, b = 14 102, c = 13 103 Å, Z = 4, D_{calc} 1 506 g cm^{-3}, D_{obs} 1 490 g cm^{-3} Of 3827 reflections (Mo-K_α radiation, Syntex $P2_1$ diffractometer), 2412 with $I > 3\sigma(I)$ were used to give, after use of the heavy atom method and least squares refinement, R_F = 13 6% and R_{WF} = 17 2% with all atoms anisotropic At this current state of refinement only gross structural features have been defined

[‡] 360 MHz ^1H n m r ($CDCl_3$ Me_4Si) δ 1 09 (3H, dd), 1 11 (3H, dd), 2 19 (1H, m), 2 36 (3H, s), 2 42 (3H, s), 2 67 (3H, m), 2 86 (1H, dq), 2 99 (1H, dq), 3 67 (1H, dd), 3 74 (2H, ddd), 3 96 (2H, ddd), 4 19 (1H, dd), 4 28 (1H, dd), and 18 75 (1H, s)

[§] Both (**2a**) and the known uni-uni-electrolyte $(Ph_3P)_2N^+Cl^-$ have molar conductivities of $1\ 1 \times 10^{-1}\ \Omega^{-1}\ cm^{-1}\ l^{-1}\ mol^{-1}$ at $5{\cdot}5 \times 10^{-3}$ M concentration in MeOH at 25 °C, demonstrating that the Cl^- in (**2a**) is extensively dissociated

[¶] Prepared by Cl atom transfer reduction of (**2g**) using 0 5 $[Cr(acac)_2]_2$ in MeOH, λ_{max} (MeOH) 520 nm, ϵ 3 7 ± 0 2 × 10^3

[**] Solutions (0 022 M) of (**2a**) in MeOH under N_2 were analysed, after addition of 1 equiv of MeO^-/MeOH, for Co^{II} [(**2c**) λ_{max} 520], $(MeO)_2CO$ [ν_{CO} (MeOH) 1755 cm^{-1}], $MeOCO_2^-K^+$ [ν_{CO} (MeOH) 1655 cm^{-1}], and MeCHO (g l c –mass spectroscopy) The yield of MeCHO represents 7 experiments (g l c on 21′ 10% 20 M Carbowax on 80/100 Chromosorb W-HP, 60 °C) in comparison to standard MeCHO–MeOH solutions E s r studies under N_2 show the expected [I(Co) = 7/2] 8 line spectrum identical to that of the independently prepared Co^{II} (**2c**)

[1] A recent review is B M Babior and J S Krouwer, *CRC Crit Rev Biochem* , 1979, **6**, 35

[2] J E Valinsky, R H Ables, and J H Fee, *J Am Chem Soc* , 1974, **96**, 4709, S A Cockle, H A O Hill, R J P Williams, S P Davies, and M A Foster, *ibid* , 1972, **94**, 275

[3] M K Essenberg, P A Frey, and R H Abeles, *J Am Chem Soc* , 1971, **93**, 1242

[4] Ref 1, p 73

[5] For recent model studies of methylmalonyl-CoA mutase see P Dowd and M Shapiro, *J Am Chem Soc* , 1976, **98**, 3724, A I Scott and K Kang, *ibid* , 1977, **99**, 1997, H Flohr, W Pannhorst, and J Retey, *Angew Chem Int Ed Engl* , 1976, **15**, 561

[6] B T Golding, T J Kemp, C S Sell, Philip J Sellars, and W P Watson, *J Chem Soc* , *Perkin Trans* 2, 1978, 839, I P Rudakova, T E Ershova, A B Belikov, and A M Yurkevich, *J Chem Soc* , *Chem Commun* , 1978, 592, G N Schrauzer, W J Michally, and R J Holland, *J Am Chem Soc* , 1973, **95**, 2024

[7] T M Vichrey, R N Katz, G N Schrauzer, *J Am Chem Soc* , 1975, **97**, 7248, R B Silverman and D Dolphin, *ibid* , 1976, **98**, 4626, K L Brown, M M L Chu, and L L Ingraham, *Biochem* , 1976, **15**, 1402, B T Golding, H L Holland, U Horn, and S Sakrihar, *Angew Chem Int Ed Engl* , 1970, **9**, 959, E A Parfenov, T G Chervyakova, M G Edelev, I M Kustanovich and A M Yurkevich, *J Gen Chem USSR* (*Engl Transl*), 1973, **43**, 2752

[8] In recent pulse radiolysis work an $HOCH_2CHOH$ radical has been generated in the presence of both B_{12}(r) and (Co^{II}) (R Blackburn, M Kyaw, G O Phillips, and A J Swallow, *J Chem Soc* , *Faraday Trans* 1, 1975, **71**, 2277) and macrocyclic Co^{II} model (H Elroi and D Meyerstein, *J Am Chem Soc* , 1978, **100**, 5540) compounds These two studies reach essentially opposite conclusions, the former finding (**1**) is not formed and the latter claiming, on the basis of spectral changes and kinetics of these changes as a function of pH, to have observed (**1**) and its rearrangement *via* a Co^{III}–π complex

[9] (a) G Costa, G Mestroni, and E de Savorgnani, *Inorg Chim Acta* , 1969, **3**, 323, (b) R G Finke, B Smith, C M Elliott, and E Hershenhart, submitted for publication, (c) G Costa, G Mestroni, and G Tauther, *J Chem Soc* , *Dalton Trans* , 1972, 450

[10] Prepared by photochemical chlorination of ethylene carbonate using Cl_2 in CCl_4 R S Newman and R W Addor, *J Am Chem Soc* , 1953, **75**, 1263

[11] The other products observed [(**2c**) (Co^{II}) and (**2h**)] suggest that, as with B_{12}(s), (R Breslow and P L Khanna, *J Am Chem Soc* , 1976, **98**, 1297) these oxidative additions may proceed *via* a one-electron mechanism

[12] (a) E G Jansen in ‘Organic Free Radicals,’ ed W A Pryor, ACS Symposia Series 69, ACS (1978), p 433, (b) E G Jansen, *Acc Chem. Res* , 1971, **4**, 31

[13] S Nigam, K D Asmus, and R L Willson, *J Chem Soc* , *Faraday Trans* 1, 1976, **72**, 2324